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TITLE: Inability of lipid A murine specific monoclonal antibody E5 to neutralize lipopolysaccharide biological activity in vitro.
AUTHOR: Bouter A.S.; Van Kessel K.P.M.; Cornelissen J.J.; Schellekens J.F.P.; Snippe H.; Verhoef J.
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Abrahamsson J; Pahlman M; Mellander L
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Thanks a lot...

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Interleukin 6, but not tumour necrosis factor-α, is a good predictor of severe infection in febrile neutropenic and non-neutropenic children with malignancy

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Abrahamsson J, Påhlman M, Mellander L. Interleukin 6, but not tumour necrosis factor-α, is a good predictor of severe infection in febrile neutropenic and non-neutropenic children with malignancy. Acta Pædiatr 1997; 86: 1059–64. Stockholm. ISSN 0803–5253

Objective: Interleukin-6 (IL6), tumor necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ) are important mediators of the inflammatory response in human infection. The aim of this study was to determine the relationship between serum levels of IL6, TNF-α, IFN-γ and CRP in febrile children with malignant disease, and relate these levels to aetiology of fever, presence of neutropenia and the effect of untreated malignancy. *Methods*: 110 febrile episodes in 70 children with malignant disease were included. Cytokine analyses were performed with sensitive immunoradiometric methods using double monoclonal antibodies. *Results*: IL6 had a sensitivity of 74% in detecting sepsis in children with fever and malignant disease. This sensitivity was not influenced by the presence of neutropenia or newly diagnosed malignancy. A positive correlation between IL6 and the CRP levels on the following day was observed ($r = .53$). TNF-α was elevated in 22% of the episodes and mean levels were significantly higher in untreated malignancy but lower in neutropenic patients. IFN-γ was elevated in 18% of cases and correlated strongly with mean TNF-α levels. *Conclusions*: IL6 is a sensitive and early predictor of bacterial infection in both neutropenic and non-neutropenic febrile children with malignancy. It is more sensitive than CRP in detecting sepsis, but the predictive value is too low to allow IL6 levels to influence initial treatment decisions in patients with granulocytopenia. TNF-α production seems to be impaired in neutropenic children and serum TNF-α cannot be employed as an indicator of bacterial infection. □ *Children, immunosuppression, infection, interferon-gamma, interleukin-6, tumour necrosis factor-alpha*

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Despite modern supportive care, infections continue to cause considerable morbidity and even mortality in children with malignant disease. Granulocytopenia, due to anti-neoplastic drug therapy, is the single most important factor contributing to the increased susceptibility of infection in these patients. Although verification of the presence of bacteria in the blood is often difficult, bacterial infection is the leading cause of fever in the child with granulocytopenia (1). Current management of febrile episodes in these patients is based on the rapid institution of empirical broad-spectrum antibiotic therapy. However, the liberal use of antibiotics has caused an increased incidence of superinfections, especially those of fungal aetiology (2).

Serum analysis of C-reactive protein (CRP) is widely used as a marker of bacterial infection but early in infection, levels of CRP are often normal or only slightly elevated. This severely limits the use of CRP as an early indicator of bacterial infection in patients with granulocytopenia (3).

Several cytokines, acting in close cooperation, are involved in the host inflammatory response to infection. Tumour necrosis factor-α (TNF-α) is a primary mediator of the inflammatory reaction and subsequent tissue damage in septic shock (4) and elevated serum levels have been demonstrated in septic shock caused by a variety of organisms (5, 6). IL6 is a multifunctional cytokine which, in bacterial infection, is released either in direct response to bacterial cell components or through stimulation by other cytokines, particularly TNF-α and interleukin-1β (7). IL6 is the major inductor of the acute-phase response in infection (8) and high serum levels of IL6 have been shown to correlate with severity of disease in human sepsis (9, 10). Several studies have shown that IL6 is often elevated in febrile neutropenia in children with malignancy (11, 12).

Interferon-gamma (IFN-γ) contributes to the induction of the acute-phase reaction, and is known to potentiate the lethal effect of TNF-α in experimental endotoxemia (13). Elevated serum levels have been documented in bacterial infection and sepsis but no consistent relation to levels of other cytokines or to outcome has been observed.

The aim of this study was to determine the relationship between serum levels of IL6, TNF-α, IFN-γ and CRP in febrile children with malignant disease and relate these levels to aetiology of fever, presence of neutropenia and the effect of untreated malignancy.

Material and methods

All patients were investigated at the paediatric oncology department in Göteborg, which is a tertiary referral centre

for children with malignant disease. The study group consisted of 70 children with malignant disease who experienced fever. Twenty-eight patients with median age 8.3 y (range 0.6–17.8) had acute lymphatic leukaemia. 10 patients with median age 2.7 y (1.0–11.5) had acute non-lymphocytic leukaemia and 32 patients with median age 9.6 y (0.6–16.3) had various solid tumours, mainly Wilms tumor, non-Hodgkin lymphoma, Hodgkin lymphoma and soft tissue or bone sarcoma. In total, 110 febrile episodes were investigated. Of these, 79 occurred in patients who had started chemotherapy, whereas 31 were in newly diagnosed and therefore untreated patients.

Fever was defined as an oral temperature of ≥ 38.5°C recorded on one occasion or as two repeated measurements of ≥ 38.0°C with an interval of 3 h. Parents were advised to bring their children to hospital, immediately fever was detected, and on arrival at the hospital the patients were rapidly subjected to a thorough physical examination. Blood was collected, through a central venous line, for analysis of haemoglobin, platelet count, white blood cell count, differential count and CRP. These tests were repeated daily until anti-bacterial treatment was discontinued. On admission, blood samples for cytokine determinations were collected into tubes containing EDTA. These samples were immediately centrifuged and frozen at –20°C. Prior to institution of antibiotic therapy a blood culture was obtained.

All but one of the patients with fever and neutropenia (absolute neutrophil count $\leq 0.5 \times 10^9$/l) were empirically treated with intravenous antibiotics. Initial treatment consisted of ceftazidim or imipenem, alone or in combination with vancomycin. Patients without neutropenia were treated with intravenous antibiotics if their CRP was $\geq 50\,\mu$g/ml or if they appeared toxic or had signs of focal bacterial infection.

In a control group of 15 healthy children, blood for cytokine determination was obtained through venipuncture.

The study was approved by the local ethics committee at the University of Göteborg and informed consent was obtained from parents and, when feasible, from the children.

TNF-α, IL6 and IFN-γ assay

All cytokines were analysed with an immunoradiometric assay (IRMA). For TNF-α, standards and samples were added to monoclonal anti-TNF-α coated tubes in the presence of a monoclonal ^{125}I-labeled anti-TNF-α directed against a different TNF epitope. After 18 h of incubation the tubes were washed with 20% Tween 20 in phosphate buffer solution and the radioactivity measured in a gamma counter (LKB Clinigamma 1272). The detection limit of the assay was 5 pg/ml. For IL6 and IFN-γ, the procedures were, with the exception of incubation times, identical and the detection limits were 5 pg/ ml and 0.8 U/ml, respectively.

For all cytokine tests intra-assay and inter-assay variations have been tested by the manufacturer yielding a coefficient of variation of 2.9–7% in the concentration ranges encountered in this study. Recovery experiments yielded a recovery of 100–110% for all cytokines. No cross-reactions between the cytokines could be detected.

For TNF-α all controls had levels below 10 pg/ml, for IL6 below 40 pg/ml and for IFN-γ below 1 U/ml. When classifying individual levels as elevated we for all cytokines used conservative estimates (IL6 $\geq$ 50 pg/ml, TNF-α $\geq$ 20 pg/ml, IFN-γ $\geq$ 2.0 U/ml).

CRP assay

Concentrations of C-reactive protein were determined by a commercially available immunoturbidimetric method (Boehringer-Mannheim, Mannheim, Germany). Reference value at our laboratory was $< 10\,\mu$g/ml. Individual CRP measurements were classified as high if $\geq 50\,\mu$g/ml.

Statistical analysis

Inferences on means were performed with multifactorial analysis of variance with Scheffé's *post hoc* test. Proportions were compared using the χ^2 test. Correlations between the cytokine and CRP levels were also analysed using simple or multiple linear regression. Cytokine values were log-transformed before ANOVA and multiple regression.

Results

The febrile episodes were divided into three groups: blood culture-proven sepsis ($n = 34$), fever of unknown origin (FUO, $n = 65$) or non-bacterial fever (NBF, $n = 11$). On clinical and laboratory grounds, patients in the NBF group were presumed to have tumour- or therapy-related fever and, despite the fact that one was neutropenic and two had CRP levels $> 50\,\mu$g/ml, none received antibiotics and all recovered uneventfully. No patient in any group had any symptoms of focal infection or evidence of the septic shock syndrome and there was no mortality.

In 56 of the 110 febrile episodes neutropenia was present

Table 1. Bacterial isolates in blood cultures from 34 children.

Bacteria	*n*	%
Staphylococcus epidermidis	16	47
Staphylococcus aureus	3	9
Pseudomonas aeruginosa	2	6
Streptococcus morbillorum	2	6
Corynebacterium difteroidun	2	6
Acinetobacter Wolffi	2	6
Escherichia coli	1	3
Klebsiella pneumonia	1	3
Serratia marcescens	1	3
Enterobacter cloacae	1	3
Streptococcus pneumonia	1	3
Neisseria mucosa	1	3
Micrococcus spp.	1	3
	34	100

Table 2. Mean cytokine levels and 95% confidence intervals (CI) in patients with culture-proven sepsis, fever of unknown origin and non-bacterial fever.

	Sepsis	FUO	NBF
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
IL6 (pg/ml)	191.0 (108.1–273.9)	140.3 (82.3–198.3)	81.8 (0–167.8)
TNF-α (pg/ml)	20.7 (9.7–31.7)	20.2 (12.6–27.8)	19.2 (2.2–36.2)
IFN-γ (U/ml)	2.7 (0.9–4.5)	2.0 (1.4–2.6)	1.1 (0.9–1.3)

and in 45 of these granulocytes were $\leq 0.2 \times 10^9$/l. Blood cultures were obtained in 93 cases and 37% (34) were positive. The bacterial species detected are given in Table 1. The incidence of bacteraemia was 39% (20/51) in neutropenic and 33% (14/42) in non-neutropenic children.

Mean cytokine levels

The mean level of each cytokine was assessed in a three-way ANOVA including the following factors: type of febrile episode, presence of neutropenia and whether chemotherapy was started or not. Table 2 gives the mean levels of the cytokines in the different groups. No significant difference in cytokine levels was observed when comparing the groups with sepsis and FUO. However, IL6 levels were significantly lower in the NBF group ($p < 0.05$).

There was no difference in mean IL6 levels in episodes with or without neutropenia, nor did IL6 levels differ between children who had or had not received chemotherapy.

As illustrated in Fig. 1, children with neutropenia had strikingly lower mean levels of TNF-α than did non-neutropenic children ($p < 0.001$). This difference was evident in all groups. Furthermore, as indicated in Table 3, TNF-α levels were significantly higher, in each of the groups, for children who as yet had not received chemotherapy ($p < 0.001$).

For IFN-γ no influence of either group, chemotherapy or neutropenia could be observed. When tested with simple linear regression no correlation between serum levels of the different cytokines was observed.

Linear regression showed a weak correlation between CRP on day 1 and IL6 ($r = 0.26$) but, as seen in Fig. 2, a much stronger correlation was found between IL6 and CRP measured on day 2 ($r = 0.53$).

Cytokines and CRP as predictors of bacterial infection

The number of episodes with high levels of cytokines on the day of admission is shown for each of the groups in Table 4. IL6 was more sensitive than any other variable in detecting sepsis and was more often elevated in the group with FUO. In the group with sepsis, 65% (13/20) of the neutropenic patients had a high IL6 as compared to 86% (12/14) of those without neutropenia. In contrast, only 29% (10/34) of patients with sepsis had a high CRP on the day of admission. Table 5 gives the sensitivity and specificity of IL6 and CRP in detecting episodes with bacteraemia.

TNF-α was elevated in 9 of the patients with sepsis,

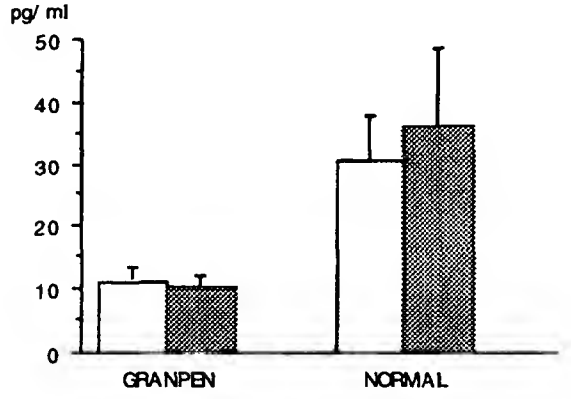

Fig. 1. Difference in mean TNF-α levels in patients with and without neutropenia. Neutropenic patients had significantly lower mean TNF-α levels as tested by ANOVA with Scheffé's *post hoc* test ($p < 0.001$). Open columns represent fever of unknown origin and filled columns fever with sepsis. Error bars indicate standard error of the mean.

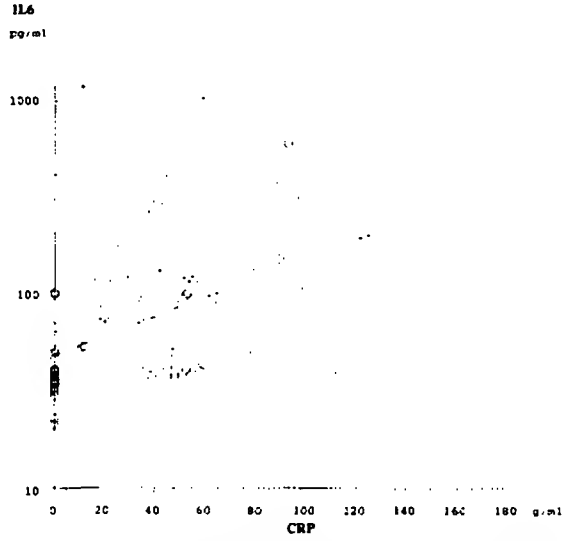

Fig. 2. The relation between the logarithm of IL6 levels and CRP levels on day 2. Linear regression showed a significant correlation ($r = 0.53$).

Table 3. Mean cytokine levels and 95% confidence intervals (within parentheses) in children who had not received (No CT) or was currently on chemotherapy protocols (On CT).

	Sepsis		FUO		NBF	
	No CT ($n = 5$)	On CT ($n = 29$)	No CT ($n = 19$)	On CT ($n = 46$)	No CT ($n = 7$)	On CT ($n = 4$)
IL6 (pg/ml)	161.9 (57.7–266.1)	196.0 (98.2–292.8)	137.5 (32.9–242.1)	141.4 (73.8–208.0)	104.5 (0–243.7)	42.1 (33.1–51.1)
TNF-α (pg/ml)	59.8 (13.2–116.4)	14.0 (5.2–22.8)	37.7 (20.9–54.5)	12.9 (5.7–20.1)	26.3 (0.7–51.9)	6.9 (1.3–12.5)
IFN-γ (U/ml)	1.7 (0.3–3.1)	2.9 (0.7–5.1)	2.0 (0.8–3.2)	1.9 (1.1–2.7)	1.1 (0.1–2.1)	1.3 (0–2.7)

namely in 4/5 newly diagnosed patients but only in 5/29 patients with sepsis and on-going chemotherapy ($p < 0.01$). A similar pattern was observed in children with FUO in whom 10/19 untreated but only 3/46 children on chemotherapy had high levels of TNF-α ($p < 0.001$). In addition, significantly fewer children with neutropenia had high TNF-α levels ($p < 0.02$).

IFN-γ was elevated in 18% of the episodes but no specific correlations with group, chemotherapy or neutropenia could be detected.

Finally, we tested interactions between the cytokines using multifactorial ANOVA. There was no interaction between IL6 and TNF-α but TNF-α levels were significantly higher in patients with high IFN-γ levels. In fact the previously found effects of granulocytopenia and chemotherapy on TNF-α levels were more significant when including IFN-γ in the model, indicating that these were independent of IFN-γ levels.

Discussion

This study demonstrates that a high proportion of neutropenic and non-neutropenic children with fever and malignancy have elevated levels of IL6. IL6 levels did not discriminate between children with sepsis or FUO whereas those with NBF had lower levels. Unfortunately, no "gold standard" of detecting bacterial or fungal aetiology is available in the evaluation of granulocytopenic infection. However, there is substantial evidence supporting that the majority of febrile episodes in neutropenic patients are caused by bacteria, despite the fact that blood cultures and/or other appropriate bacterial cultures are negative in the majority of cases (14–16).

Serum IL6 was more sensitive than CRP in detecting bacteraemia in both neutropenic and non-neutropenic children. Nevertheless, since the consequences of withholding antibiotics in a neutropenic patient with bacterial infection are so severe, and the sensitivity of IL6 was only 74% in detecting bacterial infection, we discourage the withholding of antibiotics in these patients on the basis of low IL6 levels. It is possible that IL6 analyses will help identify those patients with low-risk granulocytopenia (17) in whom intravenous antibiotics may be discontinued before granulocyte recovery.

The fact that some patients with NBF have a high IL6 can have several explanations. Since most patients with NBF were non-neutropenic, it is possible that some of these had undetected bacteraemia that resolved spontaneously. In addition, IL6 is known to be released in many inflammatory conditions of non-infectious origin. Furthermore, some patients with high IL6 levels failed to exhibit a rise in CRP levels. However, the majority of our patients commenced treatment with antibiotics within a few hours after start of fever. It is possible that some of these had an early stage bacterial infection in which the acute phase reaction was rapidly attenuated by antibiotic treatment without the production of substantial amounts of CRP.

The finding that some children with sepsis or high CRP levels on the first 3 days had low IL6 levels could depend on several factors. Although Gram-positive bacteria, in particular *S. epidermidis*, have emerged as the most commonly isolated pathogens in neutropenic fever, some of our positive cultures may represent contamination and not true

Table 4. The number of patients with high levels of IL6, TNF-α and IFN-γ on the day of admission in the different groups.

	Sepsis ($n = 34$)	FUO ($n = 65$)	NBF ($n = 11$)	All ($n = 110$)
IL6 (≥50 pg/ml)	25 (74%)	37 (57%)	1 (9%)	63 (57%)
TNF-α (≥20pg /ml)	9 (27%)	13 (20%)	2 (18%)	24 (22%)
IFN-γ (≥2 U/ml)	7 (20%)	12 (19%)	1 (9%)	20 (18%)

Table 5. Sensitivity, specificity, predictive value and validity of CRP and IL6 in the detection of bacteraemia in febrile children on the day of admission. Cut-off limits were for CRP 50 μg/ml and for IL6 50 pg/ml.

	Sensitivity	Specificity	Predictive value	Accuracy
CRP	29% (10/34)	78% (46/59)	43% (10/23)	60% (56/93)
IL6	74% (25/34)	49% (29/59)	45% (25/55)	58% (54/93)

bacteraemia. Moreover, in human sepsis, IL6 is usually detected in the early phase of infection and IL6 levels may already have returned to normal at time of sampling. Locally produced IL6, not resulting in elevated serum concentrations, may also induce CRP synthesis. Several other cytokines, such as interleukin-1β, are also capable of inducing synthesis of acute-phase proteins (18). Finally, although we found no difference in IL6 levels in untreated and treated children, previous studies have shown that TNF-α concentrations, as well as IL6 concentrations, often are elevated at diagnosis of childhood malignancy (19, 20). These cytokines interact in a complex network, including feedback inhibition, and it is conceivable that some patients are refractory to stimulation of IL6 synthesis.

Patients with or without neutropenia were equally likely to have elevated levels of IL6, suggesting that the capacity of IL6 production is not attenuated by the effects of antineoplastic drug treatment. This is consistent with other studies in febrile neutropenia in both children and adults (12, 21).

Although studies in adults have shown that TNF-α may be increased in febrile neutropenia, we only rarely observed high levels of TNF-α in febrile episodes occurring after the onset of chemotherapy (22). This may reflect the fact that none of our patients had severe toxic symptoms with complicating septic shock and, therefore, either did not produce significant amounts of TNF-α, or had only a transient increase in TNF-α, not detected at the time of blood sampling. It is also possible that repeated lipopolysaccharide stimulation, due to a chemotherapy induced disruption of the intestinal mucosa, can induce a tolerant state, in which further stimulation with endotoxin does not elicit TNF-α production (23). The presence of TNF-α-inhibitors, particularly in those children with elevated TNF-α levels at time of diagnosis could also explain the low TNF-α levels (24).

Mean TNF-α levels were consistently lower in children with neutropenia. Multifactorial ANOVA clearly demonstrated that this was an independent effect and thus was not related to the higher proportion of untreated children among the non-neutropenic individuals. Although it has been demonstrated that even severely neutropenic patients retain some capacity of TNF-α production (25), a chemotherapy-induced reduction of TNF-α producing cells is probably responsible for these lower TNF-α levels. It is possible that a lower TNF-α producing capacity in neutropenic patients, confers some protection against the detrimental systemic effects of hypercytokinemia in bacterial infections, since a lower incidence of mortality and ARDS has been seen in neutropenic adults with fever (26).

In conclusion, we have found that IL6 is an earlier and more sensitive indicator than CRP in detecting bacteraemia in children with fever and malignant disease. This suggests that IL6 can be used as a marker of bacterial infection. However, the sensitivity of IL6 in detecting bacterial infection is too low to affect decision-making regarding initial antibiotic treatment in patients with neutropenia. In addition, the predictive value and validity were similar for both IL6 and CRP. In contrast, although TNF-α and IFN-γ may be important mediators of the response to infection in these patients and exhibit an intriguing correlation to both neutropenia and malignant disease, determinations of the serum concentrations of these cytokines in febrile neutropenia seem to be of limited diagnostic value.

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Inability of lipid A murine specific monoclonal antibody E5 to neutralize lipopolysaccharide biological activity *in vitro*

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Summary

The use of anti-endotoxin monoclonal antibodies (mAbs) for the therapy of Gram-negative sepsis is controversial. Murine mAb E5, reactive with different rough and smooth lipopolysaccharides (LPS) and lipid A, has been evaluated in several experimental models and clinical trials. In the present study mAb E5 was evaluated for its capacity to neutralize toxic effects of LPS *in vitro* to understand the biologic basis for its proposed activity *in vivo*. Despite the use of high concentrations of mAb, E5 did not significantly neutralize LPS as assessed by LPS induced priming of neutrophil oxidative burst, adhesion of granulocytes to LPS stimulated endothelial cells or the release of cytokines (tumour necrosis factor (TNF) and interleukin (IL1β and IL6) from monocytes in an *ex vivo* whole blood stimulation assay. It was concluded that the proposed protective capacity of mAb E5 *in vivo* can not be explained by neutralization of the investigated endotoxin effects *in vitro*.

Key words: Endotoxin, leukocytes, endothelium, cytokines

Introduction

During Gram-negative bacteraemia and septic shock endotoxin from bacteria is released into the circulation and is held responsible for the pathophysiological features of this syndrome. Among other new therapeutic approaches, passive immunotherapy with anti-endotoxin antibodies has been studied extensively[1,2]. Most attention has been focused on antibodies to conserved epitopes in the lipid A-core-oligosaccharide region of lipopolysaccharide (LPS). Such antibodies crossreact with LPS from heterologous bacteria and may afford cross-protection against heterologous endotoxaemia or bacteraemia. Especially crossreactive mAbs with a specificity for lipid A, the toxic moiety of LPS, have been a subject of experimental and clinical studies. MAb E5, an IgM mAb of mouse origin was reported to be reactive with an extensive panel of rough as well as smooth LPS and specific for lipid A[3,4].

Experimentally, mAb E5 had been shown to decrease mortality in mice which, upon challenge with viable Gram-negative bacteria, were also treated with antibiotics[5]. Furthermore, E5 alone or in combination with antibiotics enhanced survival and reduced serum LPS and TNFα levels in neutropenic rats with Pseudomonas sepsis[6]. These studies suggest a protective effect of mAb E5.

Recently, passive immunotherapy with mAb E5 was shown to reduce mortality in a subgroup of patients with Gram-negative bacteraemia. A beneficial effect was selectively observed in those patients with Gram-negative bacteraemia who were not in shock at study entry[7]. However, in a confirmatory study, this effect could not be conclusively reproduced, despite carefully designed criteria for enrolment of patients[8]. A

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proposed mechanism of action of anti-endotoxin antibodies is to bind and neutralize circulating LPS. The present study was performed to investigate the ability of mAb E5 to neutralize the effects of LPS in several *in vitro* assays of endotoxin bioactivity.

Materials and methods

Lipopolysaccharides

J5-LPS and Re-LPS were isolated from *Escherichia coli* J5 and *Salmonella minnesota* Re595 respectively by the phenol-chloroform-petroleum-ether method as described by Galanos[9]. Smooth LPS of *E. coli* O111:B4 was extracted according to the method of Westphal[10]. All LPS preparations were dissolved in pyrogen-free distilled water at 5 mg ml^{-1} and stored at –20° C until use. Before each experiment, stock solutions of LPS were adjusted to pH 7 with triethylamine, sonicated and freshly diluted in pyrogen-free phosphate buffered saline (PBS).

Monoclonal antibodies

MAb E5 was a gift from Xoma (Xoma Corporation, Berkeley, CA) and supplied as a 2 mg ml^{-1} solution in PBS with 0.01% polysorbate 80. The murine IgM mAb E5 crossreacts with LPS from different Gram-negative bacteria and is specific for lipid A[3,4]. Different murine IgM mAbs, developed in our laboratory, were used in parallel. MAb 26–20, directed against the hydrophobic acyl residue of lipid A[11], was used as a positive control. MAb 3–12, directed against the O-antigen of O111:B4[12] and mAb 36–2, directed against *Staphylococcus epidermidis* without activity to either Gram-negative bacteria, LPS or lipid A, served as negative controls. Polymyxin B (PMB, Sigma, St. Louis, MO, USA) was used as a potent inhibitor of LPS effects.

Isolation of granulocytes

Heparinized venous human blood was obtained from healthy donors. Polymorphonuclear granulocytes (PMN) were purified by dextran sedimentation followed by Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient centrifugation. After lysis of residual erythrocytes by hypotonic shock, PMN were washed and resuspended in pyrogen-free PBS.

Priming of PMN

LPS mediated priming for enhanced release of reactive oxygen species was measured by luminol (10 µM, Sigma) enhanced chemiluminescence (CL) after stimulation with formyl-methionyl-leucyl-phenylalanine (FMLP) as described by Cornelissen[13]. LPS (100 ng) was preincubated in PBS with or without 10 µg mAb for 30 min at 37° C and used to prime PMN (2.5×10^6) for another 30 min at 37° C in a 500 µl total volume. Cells were subsequently stimulated with 1 µM FMLP (Sigma) and CL was measured continuously during 3 min at 37° C. For direct stimulation of PMN CL response, 2.5×10^6 cells were stimulated with 100 µg LPS and CL was measured during 30 min[14].

PMN adherence to endothelial cells

Human vascular endothelial cells (EC) were isolated from umbilical cord veins and cultured in tissue culture medium (RPMI, Gibco Biocult Ltd., Paisley, UK) supplemented with 20% HPS as previously described[15]. For experiments, subcultured cells from the second passage were grown to confluency in fibronectin coated 96-well plates. LPS (100 ng ml^{-1}) was preincubated in RPMI +1% HPS with or without mAbs or PMB (10 µg ml^{-1}) and subsequently the complexes were added to the endothelial monolayers for 4 h. After washing, prewarmed human PMN (2.5×10^5 per well) were allowed to adhere to the EC for 10 min at 37° C. Non-adherent PMN were gently washed away and bound cells were dissolved in 1% Triton X-100. The myeloperoxidase (MPO) content of adherent PMN was determined with o-dianisidine (Sigma) and H_2O_2, measuring the optical density (OD) at 450 nm. To determine the total MPO content of 2.5×10^5 PMN, cells were sedimented and treated in parallel. Adherence of PMN was calculated relative to the total MPO content of added PMN.

Cytokine detection in whole blood

Undiluted fresh heparinized whole blood was stimulated *ex vivo* with different amounts of Re-LPS, preincubated in tissue culture medium (DMEM, Gibco) with or without mAbs, according to the method described by Desch et al.[16]. After 5 h of stimulation, samples were centrifuged and cytokine levels were measured in the remaining supernatants, using a reference standard in each cytokine assay.

Biologically active TNF was quantitated using the actinomycin D sensitized murine cell line L-929[17]. Briefly, serial dilutions of the samples were added on top of a monolayer of L-cells. After incubation for 18 h in the presence of 1 µg ml^{-1} actinomycin D, adherent cell numbers were assayed with methylene blue. The amount of TNF was calculated from the 50% lysis points using an r-TNF reference. Amounts of immunoreactive TNFα, IL1β and IL6 were estimated using a commercially available enzyme-linked immunosorbent assay (ELISA) system (Research and Diagnostics Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions. *P* values were calculated using the Student's *t* test.

Results

Priming of PMN

Preincubation of PMN with 100 ng rough LPS resulted in an elevenfold increase (11 ± 4) in FMLP stimulated

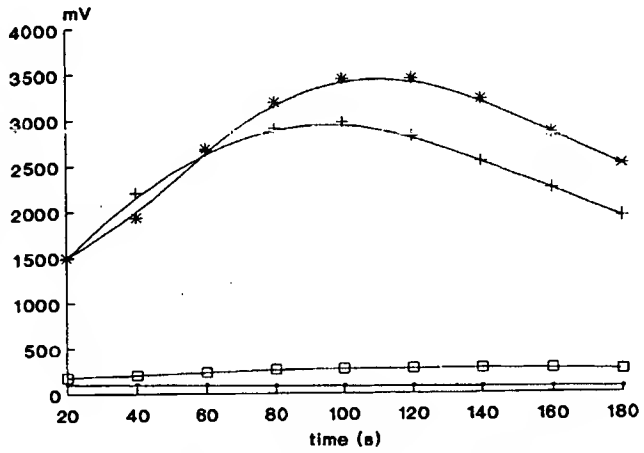

Figure 1. Inhibition of enhanced CL. PMN were primed with J5 LPS, preincubated with either E5 (–*–) or anti-lipid A mAb 26–20 (–□–), with J5 LPS in PBS (–+–) and in PBS alone (–•–). Mixtures were subsequently stimulated with FMLP and luminol enhanced CL was measured in time.

Table 1. Efficacy of mAbs in LPS primed enhanced CL of PMN*

	% Inhibition ± SEM	
mAbs	*J5-LPS*	*Re-LPS*
E5	–17 ± 6	–29 ± 14
36–2†	–25 ± 13	–37 ± 27
26–20‡	79 ± 8	80 ± 6

*PMN were primed by 100 ng LPS, preincubated with 10 µg mAbs. Results are expressed as percentage inhibition of LPS induced enhancement of CL and are the mean of at least three independent observations with PMN from different donors.
†Anti-*S. epidermidis*.
‡Anti-lipid A.

Table 2. Inhibition of PMN adherence to J5-LPS stimulated endothelial cells*

mAbs	*% inhibition ± SEM*
E5	1 ± 9
36–2†	–14 ± 13
PMB	67 ± 9

*J5-LPS (100 ng ml^{-1}) was preincubated with 10 µg ml^{-1} mAbs or PMB. Adherence of PMN was calculated by measuring their MPO content. Inhibition of LPS induced adherence was expressed as a percentage of the PMN adherence due to LPS in RPMI without mAbs. Results are the mean of at least five independent observations in triplicate.
†Anti-*S. epidermidis*.

CL response compared to buffer treated cells. Preincubation of 100 ng LPS with 10 µg mAb E5 or the non-lipid A mAb 36–2 did not prevent the LPS induced enhanced CL, whereas the anti-lipid A mAb 26–20 inhibited the response for both Re-LPS and J5-LPS by 80% (Table 1). Figure 1 shows a representative experiment of the effect of the anti-lipid A mAbs E5 and 26–20 on Re-LPS induced priming of PMN CL response. To investigate whether mAb E5 in concentrations other than 10 µg (which saturates 100 ng LPS as confirmed by ELISA) could prevent enhanced CL, a range of concentrations of both mAb E5 (1–250 µg) and LPS (1 ng–30 µg) were preincubated prior to priming and confirmed the lack of significant inhibition of the primed CL response. No inhibition of direct stimulation of the oxidative burst of PMN with 100 µg LPS preincubated with 100 µg E5 was observed, while the anti-lipid A mAb 26–20 still inhibited 74% (data not shown).

PMN adherence to endothelial cells

Incubation of EC with J5-LPS in medium for 4 h resulted in 42 ± 10% PMN adherence, compared to 8 ± 4% spontaneous adhesion to medium incubated EC. MAb E5 as well as mAb 36–2 (non-lipid A) were unable to inhibit the enhanced adherence of PMN to J5-LPS treated EC, whereas polymyxin B prevented the adherence by 67% (Table 2). None of the mAbs tested were able to prevent the enhanced PMN adherence to EC induced by Re-LPS or O111-LPS, while PMB inhibited 43% and 91% respectively (mean of two independent experiments, results not shown). A range of concentrations of mAbs and PMB (0.1–100 µg ml^{-1}) was preincubated with 100 ng ml^{-1} J5-LPS and showed concentration dependent inhibition by PMB but no significant inhibition by any mAb tested (data not shown).

Cytokine detection in whole blood

To study cytokine release by monocytes, a whole blood stimulation assay was used. Whole blood was stimulated for 5 h *ex vivo* with optimal concentrations of Re-LPS, preincubated in DMEM with or without 10 µg PMB, the non-lipid A mAb 36–2 or mAb E5 and TNF release was measured using the L-cell bioassay. Only PMB showed a significant inhibition of TNF release by 30 and 100 pg Re-LPS, up to 66%. Preincubation of LPS with mAb E5 did not result in a significant inhibition of TNF release (Table 3). To examine other cytokines released after LPS stimulation in whole blood *ex vivo*, immunoreactive human TNFα, IL1β and IL6 were determined by ELISA. MAb E5 did not show inhibition percentages different from those of the non-lipid A mAb 36–2 while PMB prevented up to 75% of the ReLPS and up to 100% of the O111-LPS induced release of all three cytokines tested. Bioassays as well as ELISA experiments with a range of concentrations of both E5 and Re-LPS failed to show a significant inhibition of ReLPS induced TNF production. No TNF could be detected after stimulation with mAbs or DMEM without LPS (data not shown).

Table 3. Efficacy of mAbs on LPS induced TNF production in whole blood

ReLPS	*DMEM*	*36–2*	*(%)*	*E5*	*(%)*	*PMB*	*(%)*
0	0.3 ± 0.2	0.4 ± 0.1		0.5 ± 0.2		<0.1	
0.1	1.4 ± 0.2	1.8 ± 0.6	(–5 ± 8)	1.0 ± 0.3	(18 ± 10)	n.t.†	
0.3	8.8 ± 5.3	9.4 ± 5.4	(8 ± 11)	3.5 ± 1.4	(32 ± 15)	0.9 ± 0.3	(53 ± 16)‡
1	9.2 ± 2.4	9.7 ± 1.8	(–6 ± 18)	8.1 ± 2.0	(7 ± 8)	2.2 ± 1.1	(72 ± 4)§
3	27.0 ± 6.1	26.4 ± 5.1	(13 ± 9)	25.7 ± 6.7	(5 ± 18)	7.8 ± 2.8	(30 ± 26)

*Re-LPS was preincubated in DMEM with or without 10 µg 36–2 (anti-*S. epidermidis*), E5 or PMB and TNF release was measured with the L-cell bioassay. Results are expressed in ng ml^{-1} TNF and are the mean ± SEM of four different donors. Inhibition percentages ± SEM are given in parenthesis and were calculated relative to TNF values in DMEM per experiment.
†Not tested.
‡$P<0.05$ (PMB vs. 36–2; two-sided).
§$P<0.01$ (PMB vs. 36–2; two-sided).

Discussion

The murine anti-lipid A mAb E5 has been shown to be beneficial in a neutropenic rat model for experimental Pseudomonas septicaemia[6] and in patients with Gram-negative bacteraemia[7]. However, the present study shows that the anti-lipid A mAb E5 was not able to neutralize biological effects of LPS significantly in several *in vitro* assays.

MAb E5 has been described to bind specifically to lipid A[3,4] and therefore this mAb was tested for efficacy to neutralize LPS-induced priming of PMN oxidative burst and production of cytokines by monocytes. These interactions are shown to be mediated by lipid A[13,18]. In addition, we studied the ability of E5 to prevent LPS-induced activation of endothelial cells for hyperadhesion of PMN.

The anti-lipid A mAb E5 did not show any significant neutralization of LPS in any of the assays studied, while another anti-lipid A mAb, 26–20[11,13] prevented LPS-induced activation in some assays.

MAbs with specificity for lipid A have been shown to be both protective and nonprotective for *in vivo* and *in vitro* neutralization of LPS biological activities. Chia et al. found that mAbs that recognize predominantly hydrophilic elements of lipid A failed to inhibit TNF secretion by mouse macrophages[19]. Others show that anti-lipid A mAbs prevented LPS stimulated TNF production by mouse macrophages *in vivo* as well as *in vitro* and protected against lethal effects of LPS[20–22]. They suggested a correlation between suppression of cytokine production and protective efficacy. Cornelissen et al.[13] have also shown that mAbs reactive with hydrophobic parts of lipid A neutralize LPS-induced priming of PMN for oxidative burst. These findings are confirmed in this study and emphasize the importance of epitope fine specificity of anti-lipid A mAbs. No data are available on the exact epitope on lipid A recognized by mAb E5 although binding could be inhibited by polymyxin B[3,4]. In the present study we could not show a significant neutralization of LPS-induced cytokine production by monocytes in a whole blood assay. Whole blood was used in order to mimic the *in vivo* situation as close as possible and to minimize activation of monocytes by cell separation. This assay has previously been used for evaluation of LPS induced TNF release by lipid IVa[23]. Although the anti-lipid A mAb 26–20 is shown to block the LPS-induced priming of PMN completely, no neutralization of cytokine production *in vitro* was observed with 26–20 or E5, despite the low LPS concentrations used and the excess of mAbs. The existence of multiple LPS receptors on different leukocytes may account for the observed inconsistency with mAb 26–20[24]. In a mouse endotoxaemia model, mAb 26–20 was shown to be protective, thereby decreasing LPS induced TNF levels *in vivo*[25]. Recently, E5 and a human anti-lipid A mAb HA-1A were shown to bind only modestly to LPS in a fluid phase assay[26] in contrast to previous reports using a solid phase assay[3,4]. E5 and HA-1A were unable to neutralize some of the biological effects of LPS as assessed by the limulus lysate assay and cytokine production from adherent monocytes and in whole blood[26].

So far, inconsistent results are found with different anti-lipid A mAbs in neutralization of biological effects of LPS *in vitro* and protection against the lethal effects *in vivo*. The variable and often conflicting results may relate to the nature of the animal model and *in vitro* test conditions as well as the antibody preparation used. Despite these contradictions, there is evidence to support the efficacy of anti-lipid A mAbs as a beneficial adjunct to conventional treatment of Gram-negative sepsis. Whether mAb E5 functions by binding to and neutralization of the toxic effects of LPS is not shown by our experiments.

Acknowledgement

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DOCUMENT NUMBER: 98010367 PubMed ID: 9350884
TITLE: Interleukin 6, but not tumour necrosis factor-alpha, is a good predictor of severe infection in febrile neutropenic and non-neutropenic children with malignancy.
AUTHOR: Abrahamsson J; Pahlman M; Mellander L
CORPORATE SOURCE: Department of Pediatrics, University of Goteborg, Sweden.
SOURCE: ACTA PAEDIATRICA, (1997 Oct) 86 (10) 1059-64.
Journal code: 9205968. ISSN: 0803-5253.
PUB. COUNTRY: Norway
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199711
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AB OBJECTIVE: Interleukin-6 (**IL6**), tumor necrosis factor-alpha (**TNF**-alpha) and interferon-gamma (IFN-gamma) are important mediators of the inflammatory response in human infection. The aim of this study was to determine the relationship between serum levels of **IL6**, **TNF**-alpha, IFN-gamma and CRP in febrile children with malignant disease, and relate these levels to aetiology of fever, presence of neutropenia. . . episodes in 70 children with malignant disease were included. Cytokine analyses were performed with sensitive immunoradiometric methods using double monoclonal **antibodies**. RESULTS: **IL6** had a sensitivity of 74% in detecting **sepsis** in children with fever and malignant disease. This sensitivity was not influenced by the presence of neutropenia or newly diagnosed malignancy. A positive correlation between **IL6** and the CRP levels on the following day was observed (r = .53). **TNF** -alpha was elevated in 22% of the episodes and mean levels were significantly higher in untreated malignancy but lower in neutropenic patients. IFN-gamma was elevated in 18% of cases and correlated strongly with mean **TNF**-alpha levels. CONCLUSIONS: **IL6** is a sensitive and early predictor of bacterial infection in both neutropenic and non-neutropenic febrile children with malignancy. It is more sensitive than CRP in detecting **sepsis**, but the predictive value is too low to allow **IL6** levels to influence initial treatment decisions in patients with granulocytopenia. **TNF**-alpha production seems to be impaired in neutropenic children and serum **TNF**-alpha cannot be employed as an indicator of bacterial infection.

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DOCUMENT NUMBER: 1994079663
TITLE: Inability of lipid A murine specific monoclonal antibody E5 to neutralize lipopolysaccharide biological activity in vitro.
AUTHOR: Bouter A.S.; Van Kessel K.P.M.; Cornelissen J.J.; Schellekens J.F.P.; Snippe H.; Verhoef J.
CORPORATE SOURCE: University Hospital, Eijkman-Winkler Laboratory, PO Box 85500,3508 GA Utrecht, Netherlands
SOURCE: Serodiagnosis and Immunotherapy in Infectious Disease, (1994) 6/1 (35-39).
ISSN: 0888-0786 CODEN: SIIDE3
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The use of anti-endotoxin monoclonal **antibodies** (mAbs) for the therapy of Gram-negative **sepsis** is controversial. Murine mAb E5, reactive with different rough and smooth lipopolysaccharides (LPS) and lipid A, has been evaluated in. . . of neutrophil oxidative burst, adhesion of granulocytes to LPS stimulated endothelial cells or the release of cytokines (tumour necrosis factor (**TNF**) and interleukin (IL1.beta. and **IL6**) from monocytes in an ex vivo whole blood stimulation assay. It was concluded that the proposed protective capacity of mAb. . .

=> s kink j a/au

L3 39 KINK J A/AU

=> dup rem l3

PROCESSING COMPLETED FOR L3
L4 21 DUP REM L3 (18 DUPLICATES REMOVED)

=> s l4 and antibod?

L5 6 L4 AND ANTIBOD?

=> d l5 total ibib kwic

L5 ANSWER 1 OF 6 MEDLINE
ACCESSION NUMBER: 2001207448 MEDLINE
DOCUMENT NUMBER: 21155963 PubMed ID: 11258548
TITLE: Oral administration of avian tumor necrosis factor **antibodies** effectively treats experimental colitis in rats.
AUTHOR: Worledge K L; Godiska R; Barrett T A; **Kink J A**
CORPORATE SOURCE: Ophidian Pharmaceuticals Inc, Madison, Wisconsin, USA.
SOURCE: DIGESTIVE DISEASES AND SCIENCES, (2000 Dec) 45 (12) 2298-305.
Journal code: 7902782. ISSN: 0163-2116.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010417
Last Updated on STN: 20010417
Entered Medline: 20010412
TI Oral administration of avian tumor necrosis factor **antibodies** effectively treats experimental colitis in rats.
AU Worledge K L; Godiska R; Barrett T A; **Kink J A**
AB . . . necrosis factor (TNF) is implicated in the pathogenesis of inflammatory bowel disease. Clinical trials indicate that intravenous infusion of anti-TNF **antibody** is an effective therapy for Crohn's disease. An oral anti-TNF therapy may be a preferred approach, reducing systemic side effects and eliminating the inconvenience and expense of administering infusions. We tested oral avian anti-TNF **antibodies** in the acute and chronic phases of a rodent colitis model. Efficacy was compared to sulfasalazine and dexamethsone. Rats with chemically induced colitis were treated orally with anti-TNF **antibody**, placebo, or comparator. Efficacy was assessed by change in colonic weight, morphology, histology, and tissue myeloperoxidase activity. Oral anti-TNF **antibody**, in both the acute and chronic phases of the model, significantly decreased all inflammatory end points and proved to be more effective than sulfasalazine and dexamethasone. Oral delivery of avian anti-TNF **antibodies** is an effective treatment of experimental colitis and may provide advantages to current parenteral anti-TNF **antibodies**.
CT Check Tags: Animal; Comparative Study; Female
Administration, Oral
***Antibodies: AD, administration & dosage**
*Chickens: IM, immunology
Colitis: CI, chemically induced
Colitis: PA, pathology
*Colitis: TH, therapy
Dexamethasone: TU, therapeutic. . .
CN 0 (**Antibodies**); 0 (IgY); 0 (Immunoglobulins); 0 (Tumor Necrosis Factor); EC 1.11.1.7 (Peroxidase)

L5 ANSWER 2 OF 6 MEDLINE
ACCESSION NUMBER: 1998234028 MEDLINE
DOCUMENT NUMBER: 98234028 PubMed ID: 9573084
TITLE: **Antibodies** to recombinant Clostridium difficile toxins A and B are an effective treatment and prevent

relapse of C. difficile-associated disease in a hamster model of infection.
AUTHOR: **Kink J A**; Williams J A
CORPORATE SOURCE: Ophidian Pharmaceuticals, Inc., Madison, Wisconsin 53711, USA.. ophidian@ophd.com
SOURCE: INFECTION AND IMMUNITY, (1998 May) 66 (5) 2018-25. Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980520
Last Updated on STN: 19980520
Entered Medline: 19980514

TI **Antibodies** to recombinant Clostridium difficile toxins A and B are an effective treatment and prevent relapse of C. difficile-associated disease in. . .

AU **Kink J A**; Williams J A

AB . . . termination of treatment. This study examined the role of both toxins in pathogenesis and the ability of orally administered avian **antibodies** against recombinant epitopes of toxin A and toxin B to treat C. difficile-associated disease (CDAD). DNA fragments representing the entire. . . cloned, expressed, and affinity purified. Hens were immunized with these purified recombinant-protein fragments of toxin A and toxin B. Toxin-neutralizing **antibodies** fractionated from egg yolks were evaluated by a toxin neutralization assay in Syrian hamsters. The carboxy-terminal region of each toxin was most effective in generating toxin-neutralizing **antibodies**. With a hamster infection model, **antibodies** to both toxins A and B (CDAD antitoxin) were required to prevent morbidity and mortality from infection. In contrast to. . .

CT Check Tags: Animal
***Antibodies, Bacterial: TU, therapeutic use**
*Bacterial Toxins: IM, immunology
Chickens
Disease Models, Animal
*Enterocolitis, Pseudomembranous: PC, prevention & control
Enterocolitis, Pseudomembranous:. . .

CN 0 (**Antibodies**, Bacterial); 0 (Bacterial Toxins); 0 (Clostridium difficile cytotoxin B); 0 (Clostridium difficile enterotoxin A); 0 (Enterotoxins); 0 (Recombinant Proteins)

L5 ANSWER 3 OF 6 MEDLINE
ACCESSION NUMBER: 92014875 MEDLINE
DOCUMENT NUMBER: 92014875 PubMed ID: 1920142
TITLE: Efficient expression of the Paramecium calmodulin gene in Escherichia coli after four TAA-to-CAA changes through a series of polymerase chain reactions.
AUTHOR: **Kink J A**; Maley M E; Ling K Y; Kanabrocki J A; Kung C
CORPORATE SOURCE: Department of Genetics, University of Wisconsin, Madison 53706.
CONTRACT NUMBER: GM22714 (NIGMS)
GM36386 (NIGMS)
SOURCE: JOURNAL OF PROTOZOOLOGY, (1991 Sep-Oct) 38 (5) 441-7. Journal code: 2985197R. ISSN: 0022-3921.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19920124

Entered Medline: 19911030

AU **Kink J A**; Maley M E; Ling K Y; Kanabrocki J A; Kung C
AB . . . plasmid harboring the altered Paramecium calmodulin gene, produces a protein judged to be calmodulin. It is recognized by a monoclonal **antibody** to Paramecium calmodulin; it migrates with the native protein at nearly the same rate in electrophoreses; and it shows a. . .

L5 ANSWER 4 OF 6 MEDLINE
ACCESSION NUMBER: 88142987 MEDLINE
DOCUMENT NUMBER: 88142987 PubMed ID: 2830512
TITLE: N-glycosylation as a biochemical basis for virulence in Leishmania mexicana amazonensis.
AUTHOR: **Kink J A**; Chang K P
CORPORATE SOURCE: Department of Microbiology and Immunology, University of Health Sciences, Chicago Medical School, IL 60064.
CONTRACT NUMBER: AI-20486 (NIAID)
SOURCE: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1988 Jan 15) 27 (2-3) 181-90.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198803
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19880329

AU **Kink J A**; Chang K P
CT Check Tags: Animal; Support, U.S. Gov't, P.H.S.
Antibodies, Protozoan: IM, immunology
Antigens, Protozoan: IM, immunology
Cells, Cultured
Glycoproteins: ME, metabolism
Glycosylation
Leishmania mexicana: GD, growth & development
. . .
CN 0 (**Antibodies**, Protozoan); 0 (Antigens, Protozoan); 0 (Glycoproteins); EC 2.7 (Phosphotransferases); EC 2.7.8.15 (UDPacetylglucosamine-dolichyl-phosphate acetylglucosamine-1-phosphate transferase)

L5 ANSWER 5 OF 6 MEDLINE
ACCESSION NUMBER: 87249180 MEDLINE
DOCUMENT NUMBER: 87249180 PubMed ID: 3036710
TITLE: Biological and biochemical characterization of tunicamycin-resistant Leishmania mexicana: mechanism of drug resistance and virulence.
AUTHOR: **Kink J A**; Chang K P
CONTRACT NUMBER: AI-20486 (NIAID)
SOURCE: INFECTION AND IMMUNITY, (1987 Jul) 55 (7) 1692-700.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198707
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19870729

AU **Kink J A**; Chang K P
AB . . . be about threefold higher in the tunicamycin-resistant variants than in the wild type, as determined by immunoprecipitation with a monoclonal **antibody** specific for this antigen. Tunicamycin treatment of the wild type and tunicamycin-resistant variants caused changes in the electrophoretic mobility of. . .

L5 ANSWER 6 OF 6 MEDLINE
ACCESSION NUMBER: 86174891 MEDLINE
DOCUMENT NUMBER: 86174891 PubMed ID: 3515177
TITLE: Expression and size heterogeneity of a 63 kilodalton membrane glycoprotein during growth and transformation of Leishmania mexicana amazonensis.
AUTHOR: Chang C S; Inserra T J; **Kink J A**; Fong D; Chang K P
CONTRACT NUMBER: AI-20486 (NIAID)
SOURCE: MOLECULAR AND BIOCHEMICAL PARASITOLOGY, (1986 Feb) 18 (2) 197-210.
Journal code: 8006324. ISSN: 0166-6851.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198605
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19970203
Entered Medline: 19860512

AU Chang C S; Inserra T J; **Kink J A**; Fong D; Chang K P

AB Our previous work by immunoprecipitation with a specific monoclonal **antibody** showed multiple, closely apposed electrophoretic bands of a major surface antigen specific to the promastigote stage of Leishmania mexicana amazonensis.. . . plus fetal bovine serum than those in serum-supplemented Schneider's medium or a defined medium; however, this is clone-dependent. Purified monoclonal **antibody** coupled to Affi-Gel 10 gave a high capacity of antigen binding, resolving four electrophoretic bands of 60-66 kDa. A 63. . . the cell surface and its capping upon the addition of rabbit anti-mouse IgG. Additional hybridomas prepared against amastigotes yielded monoclonal **antibodies** which recognized surface antigens common to both stages of the parasite.

CT Check Tags: Animal; Support, U.S. Gov't, P.H.S.
Antibodies, Monoclonal
*Antigens, Protozoan: AN, analysis
*Antigens, Surface: AN, analysis
*Glycoproteins: AN, analysis
Immunosorbent Techniques
*Leishmania mexicana: GD, growth &. . .

CN 0 (**Antibodies**, Monoclonal); 0 (Antigens, Protozoan); 0 (Antigens, Surface); 0 (Glycoproteins); 0 (Oligosaccharides)

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	23.35	23.56

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